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14. ABSTRACT

AIB1 which stands for "Amplified in Breast cancer", codes for a protein that is a member of the steroid receptor coactivator (SRC) family. AIB1 is amplified in approximately 5-10% of breast cancers and the mRNA and protein over expressed in >30% of breast cancers. AIB1 interacts with a super family of ligand activated nuclear receptors to potentiate transcriptional activity leading to upregulation of downstream target gene expression. An important finding was that an isoform of AIB1 (Δ3AIB1) is a significantly more effective coactivator of the estrogen receptor than AIB1 and is highly over expressed in human breast cancer. Prior work in our lab showed that the downregulation of overall levels of AIB1 plus Δ3AIB1, using a regulatable AIB1 directed ribozyme, resulted in reduced tumor growth in vivo. Overall, these data indicate a major role for AIB1 and its isoform Δ3AIB1 in breast cancer development and growth. However the relative roles of AIB1 versus the more highly active Δ3AIB1 in phenotypic changes in the breast has not been determined. In this investigation, we are developing a method to use siRNA directed at Δ3AIB1 in order investigate its role in breast cancer and as a possible future therapeutic approach to breast cancer.

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INTRODUCTION

Of particular interest to breast cancer was the discovery that an area of chromosome 20q, known to be frequently amplified in breast cancer, harbored the gene for AIB1. AIB1 which stands for " **A**mplified **i**n **B**reast cancer" codes for a protein which is a member of the steroid receptor coactivator (SRC) family. AIB1 is amplified in approximately 5-10% of breast cancers and the mRNA and protein overexpressed in >30% of breast cancers. AIB1 interacts with a superfamily of ligand activated nuclear receptors including the estrogen receptor (ER) and progesterone receptor (PR) to potentiate transcriptional activity leading to upregulation of downstream target gene expression. An important finding was that an isoform of AIB1 (Δ3AIB1) is a significantly more effective coactivator of the estrogen receptor than AIB1 and is highly overexpressed in human breast cancer. Prior work in our lab showed that the downregulation of overall levels of AIB1 plus Δ3AIB1, using a regulatable AIB1 directed ribozyme, resulted in reduced tumor growth *in vivo*. Overall, these data indicate a major role for AIB1 and its isoform Δ3AIB1 in breast cancer development and growth. However the relative roles of AIB1 versus the more highly active Δ3AIB1 in phenotypic changes in the breast has not been determined. Specifically, the research done with the funding of this grant will examine and compare the effects of selective reduction in the gene expression of isoform Δ3AIB1 by siRNA versus the phenotypic responses to siRNA directed at both AIB1/Δ3AIB1. Ultimately, the information from this study will be used as a basis for the development of AIB1 and Δ3AIB1 directed siRNA as a potential therapy in humans.

BODY

Task 1: **Design small interfering siRNA molecules that specifically target the nuclear receptor coactivator isoform Δ3AIB1.**

In addition to developing siRNAs to target the nuclear receptor coactivator isoform Δ3AIB1, we are now in the process of designing and developing a lentiviral plasmid system to express short-haripin RNAs to target AIB1 and Δ3AIB1. We tried targeting AIB1 with five different short-hairp RNAs as listed below. (Figure 1) Currently, we have successfully developed a lentiviral system to target and AIB1 with three of our of five of the short-hairpin RNAs we tried. Our results showed that we are able to knockdown protein levels of AIB1 in the human breast cancer cell line MDA-MB-231. (Figure 2) The lentiviral system was also able to knockdown protein levels of AIB1 in other breast cancer cell lines including MCF-7 breast cancer cell line. This lentiviral system will serve as a framework to develop a lentiviral expressed short hairpin RNAs to target the Δ3AIB1 isoform.

NCOA3 (AIB1) shRNA-lentivirus

TRCN0000019699
CCGGGCCGCATTACTACAGGAGAAACTCGAGTTTCTCCTGTAGTAATGCGGCTTTTT
Clone ID: NM_006534.2-957s1c1
Accession Number(s): NM_181659.1, NM_006534.2

TRCN0000019700
CCGGCCATACATTTAATTGCCGTATCTCGAGATACGGCAATTAAATGTATGGTTTTT
Clone ID: NM_006534.2-781s1c1

TRCN0000019701
CCGGCCTCCGCAACAGTTTCCATATCTCGAGATATGGAAACTGTTGCGGAGGTTTTT
Clone ID: NM_006534.2-4106s1c1

TRCN0000019702
CCGGCCTCTACATCTGGAGGAGTATCTCGAGATACTCCTCCAGATGTAGAGGTTTTT
Clone ID: NM_006534.2-2187s1c1

TRCN0000019703
CCGGGCAGTCTATTCGTCCTCCATACTCGAGTATGGAGGACGAATAGACTGCTTTTT
Clone ID: NM_006534.2-2740s1c1

Figure 1: We obtained the above listed five sequences for short-hairpin RNAs to knockdown AIB1 protein levels from the RNAi Consortium. This database was developed by MIT and contains a lentiviral plasmid library that expresses short-hairpin RNAs to target 15,000 human genes (http://www.borad.mit.edu/genome_bio/trc/).

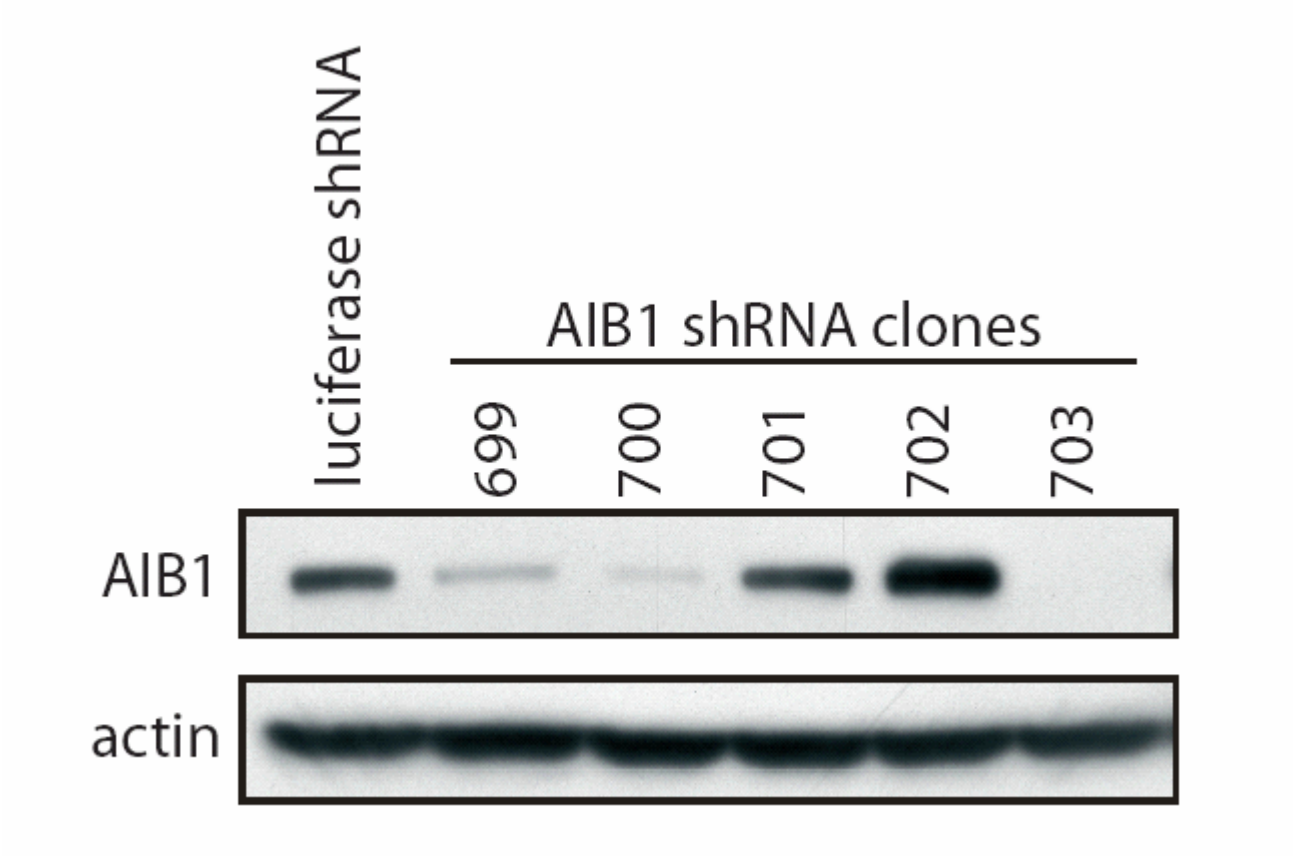

Figure 2: HEK293T cells were transfected with the individual shRNA vector, packaging vector, and envelope vector. Twenty four hours after plating, the cells were replaced with fresh media. This media or supernatant was then collected after twenty-four hours, filtered and then added to MDA-MB 231 cells. After twenty four hours, the MDA-MB 231 cells were harvested, a protein lysate was made and analyzed by western blot for AIB1 expression. Action was used as a loading control.

Task 2: **To determine if siRNA reduction of cellular levels of AIB1 of Δ3AIB1 can change the phenotype of breast cancer cell lines.** (Please see appended reprint.)

During ongoing studies of Δ3AIB1 in breast cancer, we have recently observed that AIB1 is proteasomaly degradaded in response to serum withdrawal and high cell density conditions in several immortalized cell lines. We have recently submitted a manuscript discussing these novel findings. Interestingly, in contrast to this phenomenon, **we have found that the Δ3AIB1 isoform is resistant to degradation in high cell density conditions**. (Figure 3) It would be interesting to target Δ3AIB1 by shRNA developed under Task 1 to see if can knockdown protein levels in high cell density conditions. This result may hold important implications for the study we plan to do in this task where we will specifically target Δ3AIB1 isoform.

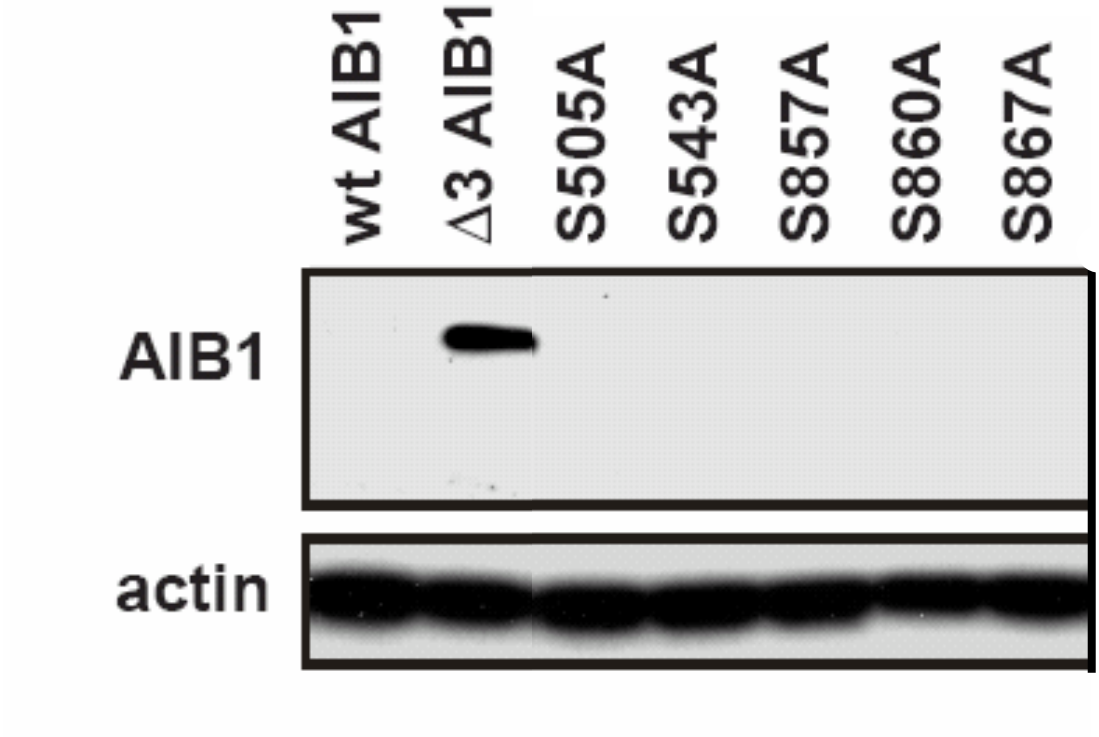

Figure 3: HEK293T cells were plated at 2.75x10^6 cells per 100mm tissue culture plate. Cells were transfected with wildtype AIB1, Δ3AIB1 isoform, or AIB1 serine phosphorylation mutants 24 hours after plating. Cells were harvested with RIPA buffer 24 hours after transfection and analyzed by Western blot for AIB1 expression. Results are representative of 2 separate experiments.

Task 3: **To determine if Δ3AIB1 siRNA is effective *in vivo*.**

We have not formally begun this task since we are awaiting the development of the Δ3AIB1 siRNA. The recent observation that we made showing that the Δ3AIB1 isoform is resistant to density dependent proteasomal degradation to which the wild-type AIB1 is susceptible is an intriguing and novel finding. We speculate that resistance to degradation diplayed by the Δ3AIB1 isoform in a high cell density condition might provide a mechanistic explanation for the finding by Reiter et al that the isoform's ability to promote transcription mediated by the estrogen or progesterone receptors was significantly greater than that of the full-length protein.[1]

KEY RESEARCH ACCOMPLISHMENTS

Development of a lentiviral plasmid system to express short-haripin RNAs to target AIB1.

REPORTABLE OUTCOMES

"E6-AP mediates regulated proteasomal degradation of the nuclear receptor coactivator AIB1/SRC-3 in immortalized cells." **Aparna Mani**, Annabell S Oh, Emma T Bowden, Tyler Lahusen, Kevin L Lorick, Allan M Weissman, Richard Schlegel, Anton Wellstein, and Anna T. Riegel", manuscript submitted to *Cancer Research*.

CONCLUSIONS

The work done to date as outlined in this report illustrates that it is possible to specifically target AIB1 using a short-haripin RNAs expressed through a lentiviral system. This gives us an initial framework from which we can design and develop short-haripin RNAs to specifically target Δ3AIB1. In addition, we plan to continue studies on the intriguing finding the isoform Δ3AIB1 is resistant to proteasomal degradation compared to wild-type AIB1.

REFERENCES

1. Reiter et al. 2001, *J Biol Chem*. V276:39736-41.